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**14. ABSTRACT**
This report describes progress in Year 2 of our 3-year award, which is designed to use animal models to understand how nicotine (ingested by Soldiers via smoking or chewing tobacco) affects vulnerability to develop post-traumatic stress disorder (PTSD). We have completed studies in which rats voluntarily self-administer nicotine to the point of dependence, receive fear conditioning (training), and are tested for fear responses 10 days later with no additional access to nicotine. This experimental design is intended to model Soldiers who use nicotine during service but later quit. We find that rats which voluntarily self-administer nicotine and are exposed to a stressor (footshock) soon after intake have abnormally reduced responses to environments previously associated with the stressor, which we term "context-potentiated startle (CPS)", but no differences in the ability to learn the association between a discrete cue (a light) and the stressor, which we term "fear-potentiated startle (FPS)". Projected to Soldiers, this suggests that self-administered nicotine is producing some anti-anxiety (beneficial) effects under these specific conditions. We also find that rats which voluntarily self-administer nicotine and are exposed to a stressor after a missed dose (i.e., during withdrawal) have abnormally persistent CPS, but no differences in FPS. Projected to Soldiers, this suggests that nicotine withdrawal is unambiguously detrimental. In Year 3 we will examine other permutations of our experimental design, including those in which access to nicotine is sustained for long periods of time between training and testing. Our findings were presented at the Substance Abuse IPR in Frederick MD on September 22-24, 2014.

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**INTRODUCTION**

Tobacco use (smoking, chewing) is prevalent in Soldiers. Nicotine has two major effects that could influence Soldier behavior and fitness: anti-anxiety (anxiolytic) effects that can have calming actions, and increases in alertness and cognitive function that can enhance aversive or traumatic memories. It is currently unknown if nicotine use increases or decreases vulnerability to the development stress-related illnesses such as post-traumatic stress disorder (PTSD). It is known, however, that people with PTSD are more likely to smoke when experiencing symptoms. These people report that smoking relieves their symptoms even though objective metrics indicate that it produces increases in hallmark signs of PTSD, such as elevated responsiveness to a startle stimulus (e.g., a white noise burst). It should be emphasized that nicotine effects on the development of PTSD is a separate question from whether or not people with PTSD smoke, and an important one because it represents an issue for which a research-driven policy change could affect Soldier health.

Animal models can offer insight on whether nicotine intake affects behavioral and molecular indicators of stress. Use of animal models enables standardization of numerous important factors, including genetics, past experiences, and levels of drug (including nicotine) intake. Perhaps most importantly, animal studies can be designed to be sensitive to beneficial or deleterious effects of nicotine. This is important because if nicotine is found to have beneficial effects, there may be safer ways to administer it to Soldiers (e.g., transdermal patch).

Our research involves a model of nicotine use (voluntary intravenous self-administration of nicotine in rats) and PTSD (fear conditioning, as reflected by fear-potentiated startle [FPS] in rats). We use FPS in rats because the same technique can be used to study PTSD in humans. It is important to emphasize that FPS in rodents is not a complete model of PTSD in humans, but it is often used to study the disorder and it does recapitulate numerous key domains—including an index trauma, persistent fearful memories, and persistent hyperarousal. Our studies have several innovative elements. In addition to the fact that that our research fills a major gap in our understanding of how nicotine might affect the development of PTSD and related behaviors, our ability to use voluntary nicotine intake in rats enables insights not possible with experimenter-delivered nicotine. In general, experimenter-administered nicotine—which can be delivered by systemic injection, by placing an animal in a passive smoke box, or by adding it to the drinking water—produces aversive responses. Most importantly, however, there is good evidence that drugs produce fundamentally different physiological effects when taken voluntarily as opposed to when it is given by the experimenter. In addition, we are able to show that the amount of nicotine voluntarily taken by our animals produces physiological dependence, as defined by the emergence of withdrawal symptoms during periods of drug abstinence. Overall, this research is intended to facilitate efforts to devise approaches that decrease new cases of stress-related illnesses in Soldiers by determining how patterns of nicotine exposure affect resilience.

This research was designed to be particularly relevant to Soldiers and thus it has numerous implications for the military. For example, if we discover nicotine has detrimental effects, it may facilitate regulation of nicotine use. In contrast, should we discover that nicotine has beneficial effects, it may be possible to devise safer ways of delivering nicotine or develop new drugs that possess only the helpful effects of the drug. In the final year of our work, we may identify a biomarker of stress vulnerability that might facilitate the development of methods to that enable better ways to match Soldier duties with biological tendencies toward stress resilience or vulnerability. The outcome of our research may also be relevant to understanding how nicotine use in civilian populations affects vulnerability to developing PTSD, particularly among individuals who may routinely be exposed to stress (e.g., law enforcement, first responders).

**BODY**

Our work will provide insight on 3 basic questions of great relevance to the military. The first question (Aim 1) is whether nicotine affects the development of conditioned fear under circumstances where nicotine self-administration is discontinued after exposure to the fear-inducing stressor. This question was addressed in studies we have now completed, and is intended to model Soldiers who are using nicotine during the time of the trauma but then remain abstinent until encountering a stressor that triggers a stress-related memory.

The second question (Aim 2) is whether nicotine affects the development of conditioned fear under conditions where nicotine self-administration is continued after exposure to the fear-inducing stressor. This is intended to model Soldiers who are using nicotine during the time of the trauma and have continued to use nicotine when encountering a stressor that triggers a PTSD-related memory. We are currently in the midst of these studies.

The third question (Aim 3) is whether there a significant relationship between nicotine effects on stress-induced activation of the transcription factor CREB in the nucleus accumbens and nicotine effects on the development of conditioned fear. We will address this question toward the very end of our 3-year award.

We are running several months behind on these studies (see Timeline, extracted from the proposal). Specifically, we estimate that we are where we thought we would be during Year 2, Quarter 3. We have explained the reasons for the delay—such as transitions in personnel, equipment failures, and slow acquisition of nicotine self-administration behavior in the rats—in our quarterly progress reports. We have addressed all of these issues to the best of our abilities, but with respect to the slow pace of the behavioral studies, we feel that there are no solutions other than acknowledging that the experiments take longer than we had hoped. We feel that the outcomes have great relevance for Soldier fitness so we do not want to implement radical changes that may have unintended consequences. Currently we do not anticipate any additional major challenges.

| Year 1 | | | | Year 2 | | | | Year 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 |
| IACUC | | | | | | | | | | | |
| Set up FPS Equipment | Aim 1 | | | Aim 2 | | | | Aim 3 | | | Manuscript Preparation |

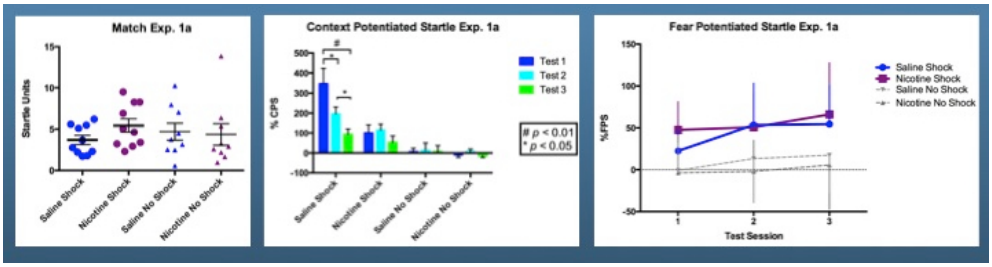
**KEY RESEARCH ACCOMPLISHMENTS**

We have accomplished all of the Year 1 goals that we described in our proposal, and have collected a data set that we feel will be of great interest to the research community. We initially presented our data at the 2013 Society for Neuroscience (SfN) conference (held in San Diego CA), and have submitted an abstract for the 2014 meeting (to be held in Washington DC on Nov 19, 2014). We intend to submit a publication describing these data before the end of 2014. In addition, the PI (Dr. Carlezon) presented these findings in-person to the Army at the Substance Abuse IPR meetings in September 2013 and 2014. We continue to collect data for Aim 2, which we envision will go into a second publication.

**REPORTABLE OUTCOMES**

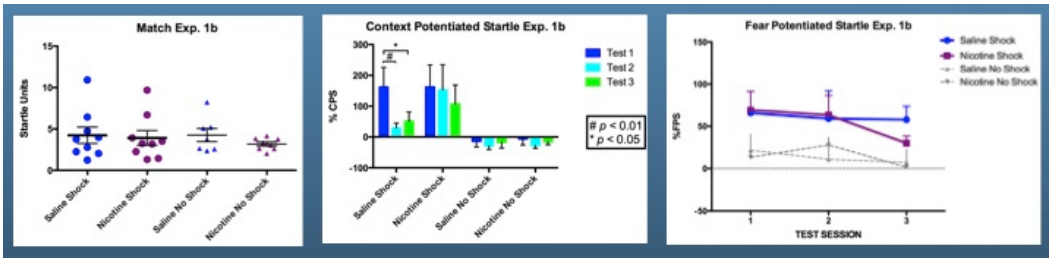
We report here the key findings of Aim 1, depicted below in Figs 1-2. During the course of performing these studies, we validated our approach and eliminated numerous factors as potential alternative explanations for our findings. There are described but not shown. First, we found that rats will develop reliable nicotine self-administration patterns when given long (12-hr) access to the drug. Previous work reported in the literature used much shorter (3-hr) access to nicotine. Second, rats will voluntarily self-administer amounts of nicotine that are sufficient to produce withdrawal during periods of no access. Specifically, we found that rats with reliable nicotine self-administration patterns displayed shaking and tremors when observed 11.5 hours their last self-administration session, a hallmark sign of dependence on the drug. Third, we found that neither nicotine nor nicotine-withdrawal caused alterations in sensitivity to the footshock, eliminating analgesia (reduced sensitivity to pain) or hyperalgesia (increased sensitivity to pain) as possible explanations for group differences in conditioned responses to the shock-associated environment (i.e., the test chamber) or the shock-associated cue (a light flash).

In Experiment 1a, we trained rats in fear conditioning immediately after their most recent self-administration session. Before the conditioning (light+shock) trials, we evaluated baseline responsiveness to the startle stimulus (a 105-dB white noise burst). We found that nicotine did not increase unconditioned responsiveness to the startle (Fig 1, left); while somewhat unexpected, this finding may indicate a tacyphylaxis (tolerance) to



the activating effects of the drug as a result of chronic self-administration. These data were used to assign the rats into shock and no-shock groups such that their mean responsiveness was roughly equivalent. Rats next received fear conditioning, consisting of 10 light+shock pairings, followed by 10 days of no treatment or access to nicotine, which is intended to model Soldiers who quit using nicotine after service. Rats were then tested in 3 sessions, each separated by 48 hr. In each test sessions, there was an initial period during which sensitivity to the startle burst alone was evaluated in the context (environment) where they previously received the light+shock pairings. In these "context-potentiated startle (CPS)" sessions (Fig 1, middle), we found that saline-treated rats show initially large CPS responses that diminish with repeated testing. In contrast, nicotine-treated rats showed abnormally low CPS throughout the repeated testing. In the subsequent "fear-potentiated startle (FPS)" sessions, during which rats were evaluated for startle in response to intermingled trials of noise-only and light+noise, nicotine had negligible effects (Fig 1, right), suggesting no effect on the ability to learn the light+shock association. These findings indicate that self-administered nicotine is producing some anti-anxiety effects under these specific conditions, which when projected to Soldiers can be envisioned as a beneficial effect of nicotine use.

In Experiment 1b, we trained rats in fear conditioning during withdrawal, 12 hr after their most recent self-administration session, modeling a "missed dose" scenario where an experienced nicotine user is not allowed access before a stressor. Otherwise, the experimental design was identical to that used for Experiment 1a. Before the conditioning (light plus shock) trials, we evaluated baseline responsiveness to the startle stimulus (a 105-dB white noise burst). We found that nicotine-withdrawal did not increase unconditioned responsiveness to the startle (Fig 2, left) the activating effects of the drug. These data were used to assign the rats into shock




and no-shock groups such that their mean responsiveness was roughly equivalent. Rats next received fear conditioning, consisting of 10 light+shock pairings, followed by 10 days of no treatment or access to nicotine, which is intended to model Soldiers who quit using nicotine after service. Rats were then tested in 3 sessions, each separated by 48 hr. In the CPS sessions (Fig 2, middle), we found that saline-treated rats show initially large CPS responses that diminish with repeated testing, similar to what we observed in Experiment 1a. In contrast, nicotine-withdrawal rats showed abnormally persistent CPS throughout the repeated testing. In the subsequent FPS sessions, nicotine-withdrawal had negligible effects (Fig 2, right), suggesting no effect on the ability to learn the association between light+shock. These findings indicate that nicotine-withdrawal is producing some anxiogenic effects under these specific conditions, which when projected to Soldiers can be envisioned as a detrimental effect of nicotine use.

We describe these findings in our 2014 SfN abstract (see Appendix). Going forward, we will report all findings (including "non-effects") each year at the annual Society for Neuroscience conference. We would like to try to package these initial findings from Experiment 1a-1b in a single publication, and to use data from Aims 2-3 for a second publication in the future. In addition, for the second year in a row we presented our findings were presented at the 2014 Substance Abuse IPR in Frederick MD (September 22-24).

One final note of progress: following our presentation at the 2014 Substance Abuse IPR, we were approached by a clinical researcher (Dr. Christine Williams) who proposed a collaborative effort to explore whether existing data from Soldiers could be "mined" to determine if nicotine use patterns can predict vulnerability to develop PTSD. While much remains to be determined with respect to feasibility, this opportunity would not have occurred if not for this award.

In Year 3 we will examine other permutations of our experimental design, including those in which access to nicotine is sustained for long periods of time between training and testing.

## CONCLUSION

We have completed studies in which rats voluntarily self-administer nicotine to the point of dependence, receive fear conditioning (training), and are tested for fear responses 10 days later with no additional access to nicotine. This experimental design is intended to model Soldiers who use nicotine during service but later quit. We find that rats which voluntarily self-administer nicotine and are exposed to a stressor (footshock) soon after intake have abnormally reduced responses to environments previously associated with the stressor, which we term "context-potentiated startle (CPS)", but no differences in the ability to learn the association between a discrete cue (a light) and the stressor, which we term "fear-potentiated startle (FPS)". Projected to Soldiers, this suggests that self-administered nicotine is producing some anti-anxiety (beneficial) effects under these specific conditions. We also find that rats which voluntarily self-administer nicotine and are exposed to a stressor after a missed dose (i.e., during withdrawal) have abnormally persistent CPS, but no differences in FPS. Projected to Soldiers, this suggests that nicotine withdrawal is unambiguously detrimental. Ongoing research examines other permutations in the experimental design, including those that allow continued access to nicotine in the period between fear conditioning and training, modeling Soldiers who continue to use nicotine after service.

## REFERENCES

Webber CJ, Adam CW, Meloni EG, Caine SB, Carlezon WA Jr (2013) Examining the effects of self-administered nicotine in an animal model of post-traumatic stress disorder. Presented at the Society for Neuroscience Conference, San Diego, CA, November 9-13, 2013.

Webber CJ, Adam CW, Meloni EG, Caine SB, Carlezon WA Jr (2014) Effects of self-administered nicotine on fear conditioning in rats. To be presented at the Society for Neuroscience Conference, Washington DC, November 15-19, 2014.

**APPENDIX**

Webber CJ, Adam CW, Meloni EG, Caine SB, Carlezon WA Jr (2014) Effects of self-administered nicotine on fear conditioning in rats. 2014 Society for Neuroscience Abstracts, in press

Nicotine can reduce stress and improve coping. It can also enhance cognitive performance and alertness, and facilitate certain forms of learning. These two actions can be conceptualized as having opposite effects on vulnerability to develop post-traumatic stress disorder (PTSD). We designed experiments to examine how nicotine self-administration (SA) followed by a period of abstinence affected the development, expression, and persistence of PTSD-like symptoms as assessed in the fear-potentiated startle (FPS) paradigm. Exaggerated startle and resistance to extinction are observed in humans with PTSD, and these signs can be studied in animal models using FPS. Experimentally naïve Long-Evans rats were allowed to self-administer nicotine (0.03 mg/inj) or saline in 12-hr (overnight) extended access sessions in standard operant conditioning chambers for a minimum of 14 sessions. This amount of access was expected to produce nicotine dependence, determined by SA of >0.7 mg/session for 4 out of 5 sessions and observable signs of spontaneous withdrawal 11.5 hrs post SA session. After meeting these criteria, separate groups of rats (N=9-10/group) were fear-conditioned at either of 2 time points: immediately after or 12 hrs after their last SA session. Fear conditioning consisted of 10 pairings of a 3-sec light co-terminating with a 1-sec 0.6 mA footshock. After fear conditioning, SA sessions were discontinued. Percent FPS (%FPS) was quantified across 3 test sessions, 48 hrs apart, 10-12 days after fear conditioning and expressed as the percent change in startle on light + startle trials over startle alone trials. In rats fear conditioned immediately after the final SA session, there were no significant differences in %FPS over the 3 test sessions between rats that had self-administered nicotine or saline. However, during test session 1, nicotine-treated rats had lower responsiveness to startle alone than those treated with saline. In rats fear-conditioned 12 hrs after the last SA session, there were no differences in %FPS, but nicotine-treated rats had higher responsiveness to startle alone during test session 1. These findings may indicate that rats self-administering nicotine immediately prior to fear conditioning show signs of protection from exaggerated responses to the startle stimulus, whereas rats conditioned during nicotine withdrawal show signs of vulnerability. This work provides the basis for exploring the effects of nicotine SA on the development and persistence of fear in rats with continued access to nicotine after fear conditioning, and may ultimately provide deeper insight on how nicotine use affects vulnerability to stress-related illnesses.

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